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Review Article

Conservation of natural enemies of *Diaphorina citri* and their impact on Huanglongbing: Analysis and perspectives

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ABSTRACT

Diaphorina citri is the main vector of *Candidatus* Liberibacter asiaticus (*C*Las), the causative agent of HLB, the most devastating citrus disease. Although the importance of biological control is recognized, insecticides are the main control tool used. Its use in citrus should be cautious, as it could interfere with the biocontrol of exotic pests already present. *Diaphorina citri* has a wide range of natural enemies; however, only the parasitoid *Tamarixia radiata* and some species of entomopathogenic fungi are used inundatively. Although the main predators of the vector occur naturally, few studies address their conservation *in situ*. This review supports the idea that the conservation of natural enemies should be the basis of the integrated management of *D. citri* and *C*Las. The conservation of alternate hosts, the inclusion of nectar plants, *in situ* conservation of parasitoids, and the autodissemination of entomopathogenic fungi are proposed. Studies carried out on conservation of natural enemies of *D. citri* and related pests, their probable impact on the disease, and prospects for implementation in Mexico are analyzed and discussed. The proposed strategies could enhance not only the biological control of *D. citri*-*C*Las, but also the autoregulation of citrus pests in general.

Keywords. Asian citrus psyllid, biocontrol, *Tamarixia radiata*, entomopathogenic fungi, conservation

INTRODUCTION

With over 600,000 hectares and a production exceeding 8 million tons in 2021, Mexico stands as one of the leading citrus producers. The economic contribution reached approximately 35 million pesos (SIAP, 2022), underscoring the significance of these crops within the country's economic sector. Their production spans across 24 out of the 32 states comprising Mexico, from the Yucatan Peninsula to Tamaulipas along the Gulf of Mexico, and from Chiapas to Sonora and the Baja California Peninsula along the Pacific (Salcedo-Baca *et al*., 2010; García-Ávila *et al*., 2021).

However, citrus crops face a plethora of phytosanitary challenges globally. Unlike other crops, successful cases of classical biological control occur within citrus cultivation. For instance, in Mexico, the cottony cushion scale (*Icerya purchasi*) is managed through the ladybird beetle *Rodolia cardinalis* and the citrus blackfly (*Aleurocanthus woglumi*), regulated by the parasitoid *Encarsia perplexa*, among others (Trevor *et al.,* 2013).

This distinctive characteristic renders citrus cultivation unique. Unlike in other crops where chemical control forms the cornerstone of pest management, pesticides in citrus cultivation must be handled with greater caution. Their misuse could disrupt the achieved ecological balance and potentially lead to the resurgence of significant pests (Qureshi and Stansly, 2009), as mentioned above.

In recent years, Mexico has witnessed new invasions of exotic pests, prominently the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Leviidae), which along with *Trioza erytreae* (Hemiptera: Triozidae), serves as a vector for *Candidatus* Liberibacter asiaticus (*C*Las), the causal agent of Huanglongbing (HLB). Following the psyllid's introduction in 2002, HLB was first documented in 2009 in Yucatan. Since then, the disease has spread to various regions, encompassing 24 citrus-producing states (García-Ávila *et al*., 2021). The risks posed by the *D. citri-C*Las pathosystem in Mexican citriculture (Salcedo-Baca *et al.*, 2010) prompted the development of integrated management strategies, highlighting chemical control alongside complementary biological approaches (García-Ávila *et al*., 2021).

In Mexico, the psyllid boasts a diverse array of natural enemies (NE), including one of its primary parasitoids, *Tamarixia radiata* (Hymenoptera: Eulophidae) (Cortez-Mondaca *et al.*, 2010; Mora-Aguilera, 2013). Although widely distributed in Mexican citrus zones, levels of parasitism remain insufficient (3-26%) for the natural regulation of the pest and *C*Las (Halbert and Manjunath, 2004; García-Ávila *et al*., 2021). To enhance biological control (BC) of *D. citri* and mitigate *C*Las dispersal risks, the Mexican government has endeavored to establish a program of inundative biological control (IBC) utilizing the parasitoid *T. radiata* in citrus regions across the country (García-Ávila *et al*., 2021). Despite scientific literature stressing the importance of conserving NE in *D. citri* management, research in this realm remains scarce globally (Qureshi and Stansly, 2009; Irvin *et al*., 2021; Irvin and Hoddle, 2021), with documented studies in Mexico being nascent (Godoy-Ceja and Cortez-Madrigal, 2018).

Under the premise that integrated pest management (IPM) should rely on NE conservation, and insecticide application should occur only upon reaching an economic threshold (ET), this paper aims to analyze the status of *D. citri-C*Las pathosystem management globally, particularly in Mexico. Based on this, it proposes conservation strategies extending beyond *D. citri* biological control to encompass the regulation of other citrus phytophagous (including pests).

The citrus agroecosystem in Mexico

Cultivated citrus traces its origins back to Southeast Asia, where its evolutionary journey spans roughly 8 million years (Ibanez *et al.,* 2015). Recognizing the geographical birthplace of a crop holds pivotal importance in combating exotic pests, often necessitating the exploration and importation of natural enemies from their native habitats. Over time, the citrus industry in Mexico has contended with a spectrum of exotic pests, some of which have been effectively managed through classical biological control methods (Trevor *et al.,* 2013).

In Mexico, citrus cultivation sprawls across 24 of the nation's 32 states (García-Ávila *et al.,* 2021). These regions boast diverse soil compositions, climatic variations, and cultural influences, all of which profoundly shape the design and management of the agroecosystem, including the dynamics of the *D. citri-C*Las pathosystem. Consequently, factors like wind patterns and orchard distribution have contributed to variations in the severity of Huanglongbing (HLB) outbreaks across different citrus regions, such as those along the Mexican Pacific versus the Yucatan Peninsula (Mora-Aguilera, 2013).

Similar to their counterparts elsewhere, Mexican citrus growers exhibit distinct characteristics, necessitating consideration of their typology. This entails understanding their profiles based on factors like land holdings, technological adoption, and educational backgrounds (Rosales-Martínez *et al.,* 2018). Such insights could facilitate tailored programs, including initiatives for conserving natural enemies. Consequently, the socioeconomic and cultural contexts of small-scale producers influence plantation features, ranging from mono-crops with weed tolerance (common in the Yucatan Peninsula) to intricate polycultures like the coffee-plantain-citrus system observed in certain regions of Veracruz (Aguilar *et al*., 2014). These agricultural models contrast starkly with extensive monocultures found in other parts of the country, such as Huimanguillo, Tabasco. Such variations extend to management practices, notably pesticide use, which is pivotal for implementing Conservation Biological Control (CBC) strategies (Gurr and You, 2016).

While some citrus plantations benefit from irrigation systems, the majority of Mexican citrus farming (63%) relies on rainfed agriculture (García-Ávila *et al.,* 2021). Nonetheless, certain regions harbor conditions conducive to thriving populations of natural enemies. For instance, in Rovirosa, Quintana Roo, high relative humidity has fostered natural epizootics of the fungus *Hirsutella citriformis* in *D. citri* (Mora-Aguilera *et al*., 2017), underscoring the potential of entomopathogenic fungi (EPFs) as crucial tools for managing the *D. citri-C*Las complex in such areas.

The Citrus-*Diaphorina citri-C*Las pathosystem

In 2002, *D. citri* was introduced to Mexico and is now distributed across all citrus-growing areas of the country (García-Ávila *et al*., 2021). Apart from the direct damage it causes to citrus, *D. citri* serves as a vector for Gram-negative α-Proteobacteria of the genus *Candidatus* Liberibacter spp., the causal agent of HLB (Galdeano *et al*., 2020). Three species are involved: *C*L. africanus, *C*L. americanus, and *C*L. asiaticus, with the latter being the most prevalent worldwide (Thakuria *et al.,* 2023). HLB damages and losses vary between countries, regions, and hosts. For example, in Brazil, losses in sweet orange fluctuated from 17.5-42%, while in Mexico, losses vary according to the crop and region, ranging from 17% for Persian lime in Yucatan to 62% for Mexican lime in Colima (Mora Aguilera *et al.*, 2014). In a scenario of low HLB intensity in Mexico, losses of 1.84 million tons of citrus are estimated, representing 25% of the national production, with losses of 4 million workdays (Salcedo *et al*., 2010).

The concept of an "epidemic" as an imbalance between populations can be applied to the citrus-*D. citri-C*Las pathosystem, where knowledge of its biology and ecology can make the difference between the success or failure of management programs. As a guide, the concept of an "epidemiological system" could be applied, conceived as an open system of n-factors operating in time and space to determine the incidence of an epidemic (Figure 1) (Mora-Aguilera *et al.,* 2017).

For *D. citri*, the optimal temperature fluctuates between 25-28 °C, with development ceasing between 10 and 33°C (Liu and Tsai, 2000). Females cease oviposition when temperatures remain at 34 °C for five consecutive days; once temperatures decrease, adults resume oviposition (Skelley and Hoy, 2004). Conversely, *C*Las does not tolerate temperatures >35 °C (Thakuria *et al*., 2023). The number of eggs per *D. citri* female decreases when relative humidity remains below 40% (Skelley and Hoy, 2004).

Moreover, humidity indirectly affects *D. citri* by favoring the prevalence of EPFs that reduce insect populations (Aubert, 1987). An interaction between light rainfall and high temperatures indirectly favored *D. citri* populations by promoting greater leaf flush in trees, a factor directly correlated with vector populations and HLB incidence (Leong *et al.,* 2020). Wind is another key factor in *D. citri* and *C*Las dispersion. It has been documented that the psyllid can fly up to 2.4 km in the absence of wind. In the presence of wind, some estimates suggest the insect could disperse up to 4 km (Mora-Aguilera, 2013) or even 470 km (Stelinski, 2019).

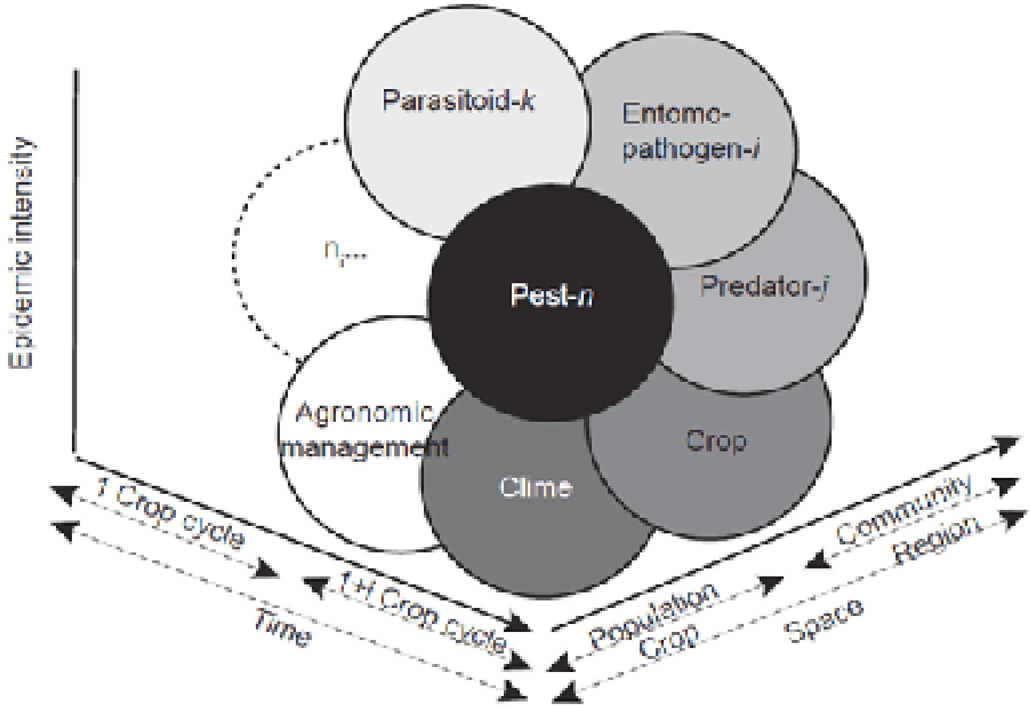

Figure 1. Epidemiological System defined by the interaction of factors that determine an epidemic process: pest species, entomopathogen, predator, and parasitoid, crop, agronomic management, climate, and any other specific factor (n_i), all of which operate at different spatial-temporal levels (Adapted from Mora-Aguilera *et al*., 2017).

Under such circumstances, wind currents were one of the factors favoring the rapid spread of HLB in Mexico (Mora *et al.,* 2013).

Overwintering adult *D. citri*, especially females, have a longer survival time (8-9 months) and congregate on the undersides of leaves. Nymphs are sedentary, but newly emerged individuals may migrate to neighboring shoots or move within the same shoot, but never to mature leaves (Yang *et al.,* 2006). An aspect of ecological interest for vector and *C*Las management is that both males and females mate multiple times with different partners, and there is evidence that the female emits a sexual pheromone to attract her mate (Wenninger and Hall, 2008). Egg production in *D. citri* varies depending on the host. For example, Tsai and Liu (2000) obtained an average of 857.9 eggs on grapefruit, while Nava *et al*. (2007) recorded 348.4 eggs on *Murraya paniculata*.

Diaphorina citri eggs are exclusively laid on vegetative shoots, and nymphs only develop on them; thus, their population fluctuation is positively correlated with these phenological periods (Leong *et al.,* 2020). The greatest damage from *D. citri* usually occurs during autumn leaf flush (Yang *et al.,* 2006; Godoy-Ceja and

Cortez-Madrigal, 2018). Under tropical conditions such as those in India, up to nine generations can occur. Based on temperature, favorable psyllid days, and planted area, estimates for Mexico suggest anywhere from 15 to 34 annual generations (Díaz-Padilla *et al.,* 2014). *Diaphorina citri* is strongly attracted to the color yellow (Patt *et al.,* 2015).

The relationship between *D. citri* and *C*Las is of a propagative-circulative nature (Galdeano *et al*., 2020), and once the pathogen is acquired, the insect remains infective throughout its life; however, transovarial transmission has not been recorded (Halbert and Manjunath, 2004). According to Inoue *et al.* (2009), insects that acquire the bacterium in the adult stage show poor efficiency as vectors; conversely, those that acquire the pathogen in the nymphal stage are more efficient as vectors. The low concentration of bacteria in the adult insect led to low transmission to the plant; however, when the psyllid feeds in the nymphal stage on an infected plant, the bacteria concentration increases significantly, and transmission occurs in up to 67% of healthy plants. The incubation period of *C*Las in the host can be up to six months (Aleman *et al.,* 2007).

It has been proven that *C*Las requires amino acids obtained from the host plant and from *D. citri*. There is ample evidence that *C*Las can manipulate the behavior and performance of the vector, thus increasing the transmissibility and spread of the pathogen. This occurs both within the vector and through intermediation of the infected plant. Positive vectors exhibit higher fertility, fecundity, and developmental rates; however, survival is reduced. Nevertheless, *C*Las favors the increase in positive populations of *D. citri* (Yamada *et al.,* 2019).

Other intriguing aspects of the citrus-*D. citri*-*C*Las relationship include the fact that positive adult insects exhibit greater flight capability than negative psyllids. Their preference for volatiles in diseased plants is evident; however, once infected, the insects disperse towards healthy plants (Yamada *et al.,* 2019). This, coupled with the females' avoidance of infested shoots, facilitates disease spread. The psyllid is initially distributed in clusters, then randomly, and once again in clusters (Leong *et al*., 2020). *C*Las manipulation extends even to mating; for instance, *D. citri* males showed greater attraction to positive females than to healthy ones. Additionally, the parasitoid *T. radiata* is primarily attracted to diseased plants (Yamada *et al*., 2019).

The psyllid's immune system is also altered by *C*Las. In positive adults, the concentration of oxidases and esterases is significantly reduced. These enzymes are crucial for insecticide detoxification; hence, positive insects are more susceptible to insecticides, including entomopathogenic fungi (Yamada *et al*., 2019). This has practical implications for NE conservation; for example, using entomopathogenic fungi or minimal doses of insecticides targeted at positive insects would cause less harm to NE.

Like other vectors, *D. citri's* immune system is incomplete; thus, the insect's associated endosymbionts contribute to the production of defense metabolites against invaders, such as *C*Las and NE (Yamada *et al.,* 2019). In positive psyllids, the production of "diaphorin," a polyketide with toxic effects on insects, increased; therefore, it could limit the activity of some psyllid NE, such as the coccinellid *Harmonia axyridis* (Galdeano *et al*., 2020). No research has been conducted to elucidate these interactions, such as levels of parasitism by *T. radiata* on positive psyllids.

For *D. citri*, 59 host species from the Rutaceae family have been recorded (Halbert and Manjunath, 2004). Additionally, the psyllid can feed on *Celtis* spp. and *Solanum tuberosum* (Stelinski, 2019). Recently, *Cordia myxa* (Boraginaceae) was reported as a host for *D. citri* (Arshad *et al*., 2019), suggesting that the psyllid (and probably *C*Las) may have previously unrecorded hosts, particularly in regions where the *D. citri*-*C*Las complex has been recently introduced.

Grafting experiments have demonstrated the susceptibility of nearly all cultivated citrus to *C*Las. *Citrus sinensis* is typically the most susceptible species, while *C. aurantiifolia* is the most tolerant (Thakuria *et al*., 2023). Ornamental species of *Murraya* spp. are also hosts for *C*Las. Although *M. paniculata* can act as a bridge host in *C*Las dissemination, the significant decrease in bacterial titer after five months suggests that this species is of little importance as a *C*Las host (Damsteegt *et al*., 2010). In contrast, *M. koenigii* showed tolerance to *C*Las, suggesting its use as a trap plant (Beloti *et al.,* 2018).

Moreover, *C*Las was successfully transmitted via *Cuscuta* spp. to plants outside the Rutaceae family, such as *Catharanthus roseus* (Apocynaceae) and different solanaceous plantas. In *Pithecillobium lucidum* (Fabaceae) *C*Las was recorded even though no psyllids were recorded (Thakuria *et al.,* 2023). Additionally, *C*Las was detected via PCR in *Cleome rutidosperma* (Capparaceae), *Pisonia aculeata* (Nyctaginaceae), and *Trichostigma actandrum* (Phytolaccaceae) without records of psyllids or HLB symptoms (Brown et al., 2014).

In 2010, adult *D. citri* were recorded on glandular trichomes of *Sicydium tamnifolium* (Cucurbitaceae) on *M. paniculata* plants in Chetumal, Q.R., Mexico. This species is distributed only along the Gulf of Mexico and Chiapas (Lira, 2004). Although its role as a host for *D. citri* is unknown, these preliminary observations suggest that *S. tamnifolium* could be utilized as a trap plant for *D. citri* and other pests. However, it is necessary to elucidate the pros and cons of its use, such as interference with biological control, crop interference, potential pest reservoir, and economic considerations.

Management of *Diaphorina citri*-*C*Las

Once *C*Las infects its host, it is difficult to eradicate, despite numerous studies demonstrating control possibilities, such as through cultural, chemical, and biological control; thermotherapy, defense inducers, and genetic manipulation for resistance (Thakuria *et al*., 2023). However, in practice, HLB management includes preventive measures, including the production of healthy plants (Yang *et al*., 2006), eradication of diseased plants, and legal control (García-Ávila *et al.,* 2021), but primarily through vector control (Yang *et al*., 2006; García-Ávila *et al*., 2021). Although integrated management of *D. citri* is proposed, greater attention is focused on chemical control (Qureshi and Stansly, 2007; Parra and Coelho, 2019), with biological control only being complementary (Etienne *et al*., 2001; Salcedo-Baca *et al.,* 2010). However, when *C*Las is present in commercial orchards, chemical control is recommended (Parra and Coelho, 2019; García-Ávila *et al.,* 2021).

Following the introduction of the *D. citri-C*Las complex in Brazil, insecticide applications increased by over 600% (Parra *et al.,* 2016). Seen in this light, chemical control could have a detrimental impact on the environment and on natural enemy populations (Qureshi and Stansly, 2009; Monzó and Stansly, 2020). For disease vectors, the ET is practically the absence of pests. For instance, the transmission rate of *C*Las by adult *D. citri* to *Citrus depressa* plants was estimated at 8.5% when one adult was introduced per plant, with a two-week inoculation access period (Ukuda-Hosokawa *et al.,* 2015). Only a few ET have been proposed for its control; for example, in Florida, five adults and three nymphs per flush are suggested (Halbert and Manjunath, 2004), or 0.2-0.7 adults per flush and 0.2 adults per trap (Monzo and Stansly, 2015). In Mexico, a proposed ET is one adult per trap (Yzquierdo-Álvarez, 2021).

Diaphorina citri has a wide range of natural enemies, including predators, parasitoids, and entomopathogens (Yang *et al.,* 2006; Qureshi and Stansly, 2007), which presumably should also reduce disease incidence and prevalence.

Entomophagous. Records worldwide document 95 arthropod species (spiders and insects) across nine orders and 23 families (Kondo *et al.,* 2015), with varying impacts on *D. citri*. For instance, in Saudi Arabia, the Order Araneae contributed up to 33.6% to vector reduction (Halbert and Manjunath, 2004). In Florida, predators contribute up to 100% to *D. citri* control (Qureshi *et al.,* 2009). These include species of Araneae, Hemerobiidae, Syrphidae, and Anthocoridae, with lady beetles such as *H. axyridis*, *Olla v-nigrum*, and *Cycloneda sanguinea* being notable (Michaud, 2004). In Colombia, 16 natural enemy species from six families and five orders affecting *D. citri* have been recorded (Kondo *et al.,* 2015). Conversely, extensive insecticide applications in Brazil limited the impact of predators on psyllid biological control (Parra *et al.,* 2016). In Mexico, 20 species of entomophagous insects have been recorded, including families Coccinellidae, Chrysopidae, Anthocoridae, and Vespidae, with the first two being more prevalent. Additionally, entomopathogenic fungi such as *Beauveria bassiana* and *Hirsutella citriformis* have been isolated from *D. citri* (Table 1).

Table 1. Natural enemies associated with *D. citri* in citrus orchards in Mexico.

Family/Species	Reference
Coccinellidae	
H. axyridis	Mora-Aguilera, 2013
O. v-nigrum	
C. sanguinea	Cortez-Mondaca *et al*., 2010
Chilocorus cacti	Mora-Aguilera, 2013
Hippodamia convergens	
Scymnus sp.	
Arawana sp.	Lozano-Contreras y Jasso-Argumedo, 2012
Azya orbigera	
Delphastus sp.	
Zagloba sp.	
Chrysopidae	
Ceraeocrhysa sp.	Lozano-Contreras y Jasso-Argumedo, 2012
C. cubana	
C. claveri	
C. valida	
C. everes	
Chrysoperla rufrilabris	Cortez-Mondaca *et al*., 2010
Ch. comanche	
Chrysoperla sp.	Mora-Aguilera, 2013
Anthocoridae	Cortez-Mondaca *et al*., 2011
Orius spp.	
Eulophidae	
T. radiata	
Encyrtidae	
Diaphorencyrtus spp.	
Globorotallidae	Mora-Aguilera *et al*., 2017
H. citriformis	
Clavicitaceae	
B. bassiana	

The primary focus of biological control against *D. citri* has been the introduction of its parasitoids, *T. radiata* and *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae) (Halbert and Manjunath, 2004), although the former has received more attention. However, parasitism levels have been variable, ranging from 28.9-36.4% in Taiwan (Chiu *et al*., 1988) to 100% in Reunion Island, France (Etienne *et al.,* 2001).

In Florida, *T. radiata* has been a relatively minor mortality factor for *D. citri*, with levels below 20% in spring-summer, but reaching 39-56% in autumn (Qureshi *et al.,* 2009). In Puerto Rico, Pluke *et al.* (2008) reported parasitism levels of 70-100%, while in Sinaloa, Mexico, natural parasitism rates of up to 59.6% were recorded (Cortez-Mondaca *et al*., 2010). The effectiveness of *T. radiata* is strongly limited by the presence of hyperparasitoids (Aubert, 1987), pesticide use (Diniz *et al*., 2020), absence of hosts in critical periods (Leong *et al*., 2020), and lack of nectar and pollen (Irvin and Hoddle, 2021).

Due to the variability in *T. radiata* parasitism, the greatest effort has been focused on mass rearing and release (Parra *et al.,* 2016; Flores and Ciomperlik, 2017; García-Ávila *et al.,* 2021). However, in commercial orchards, this strategy is incompatible with pesticides (Diniz *et al*., 2020). Thus, biological control is only practiced in backyard orchards, urban areas, and abandoned plantations, with scientific evidence of reducing *D. citri* and HLB incidence in commercial orchards (Parra *et al.,* 2016; Diniz *et al.,* 2020; García-Ávila *et al.,* 2021). For example, in Texas, USA, after releasing *T. radiata*, vector reduction exceeded 91% (Flores and Ciomperlik, 2017). In countries like Brazil, parasitism levels reached 72.5% after parasitoid release (Parra *et al*., 2016). In Mexico, it is mentioned that natural parasitism by *T. radiata* (3-26%) increased to 85% after mass releases (García-Ávila *et al*., 2021).

Entomopathogenic fungi. Prominent among these species are *H. citriformis*, *B. bassiana* (Padulla and Alves, 2021), *Isaria fumosorosea* (Gallou *et al.,* 2016), and *Metarhizium anisopliae* (Ayala-Zermeño *et al.,* 2015). However, only *H. citriformis*, *B. bassiana*, and *I. fumosorosea* (=*Paecilomyces fumosoroseus*) have been isolated from the insect (Halbert and Manjunath, 2004; Meyer *et al*., 2008; Mora-Aguilera *et al.,* 2017). These entomopathogenic fungi have mainly been evaluated using inundative strategy (*i.e*., spraying); in Brazil, their field application achieved mortality levels of 57.8% to 96.1%, directly correlated with humidity and inversely with temperature (Saldarriaga-Ausique *et al*., 2017).

In Mexico, EPFs represent a significant tool against *D. citri* and *C*Las in commercial citrus orchards (García-Ávila *et al*., 2021). After assessing *Cordyceps bassiana*, *M. anisopliae*, and *I. fumosorosea* in Colima, Mexico, the highest mortality in nymphs (60%) and adults (50%) was achieved with *C. bassiana* (Lezama-Gutiérrez *et al.,* 2012). Under field conditions, *I. javanica* (=*fumosorosea*) achieved mortality rates of 66-81.8% (Gallou *et al.,* 2016).

EPFs can also colonize plant tissues. Bamisile *et al.* (2021) assessed the endophytic capability of *B. bassiana* and *I. fumosorosea*, finding that only the former colonized citrus tissues. Seven days after inoculation, mortality in *D. citri* adults ranged from 10% to 15%, and there was a significant reduction in egg deposition by females feeding on treated plants.

Beyond biological control of the *D. citri*-HLB pathosystem: the conservation of natural enemies

There's a prevailing notion that once *C*Las takes hold, the biological control (BC) of *D. citri* becomes inconsequential for managing HLB (Helbert and Manjunath, 2004; Yang *et al.,* 2006). This might hold true if we consider BC as simply mirroring chemical control: aiming to drastically reduce pest populations. However, shifting away from the conventional and atomistic paradigm of pest management and its derived inundative biological control (IBC), we can discern a natural phenomenon of population self-regulation so potent that conservation biological control (CBC) should form the bedrock of IPM.

CBC encompasses all measures aimed at environmental modification and pesticide management to favor the conservation and proliferation of beneficial organisms (Gurr and You, 2016). Despite being the linchpin of biological control, CBC garners minimal attention (Cortez-Madrigal and Gutiérrez-Cárdenas, 2023). This is largely due to modern agricultural practices being geared towards productivity rather than the preservation of beneficial organisms. Various strategies are proposed for their conservation, including pesticide management, devices for *in situ* conservation and augmentation of parasitoids (Kehrli *et al.,* 2005), provision of alternate food sources such as nectar, pollen (Gurr and Wratten, 1999; Gurr and You, 2016) and alternate hosts (Cortez-Madrigal and Gutiérrez-Cárdenas, 2023).

When beneficial organisms lack alternative food sources in a crop, they migrate to areas where sustenance is available, undercutting the presence of functional populations of beneficial organisms (Cortez-Madrigal and Gutiérrez-Cárdenas, 2023) or delaying their arrival to such an extent that BC becomes ineffective (Doutt and Nakata, 1973). This scenario can be altered by redesigning the agroecosystem to support the permanent establishment of parasitoids, predators, and entomopathogens before the pest appears, not only of insects and mites but of the entire spectrum of organisms involved in population self-regulation within ecosystems; a holistic view of CBC: "beyond biological control" (Cortez-Madrigal and Gutiérrez-Cárdenas, 2023).

Though the economic benefits of CBC in regulating *D. citri* populations have been validated (Monzó and Stansly, 2020), there's a dearth of documented studies (Irvin *et al*., 2021; Irvin and Hoddle, 2021). Some conservation strategies for beneficial organisms evaluated in *D. citri* or related pests include nectar-rich flora, alternate hosts for beneficial organisms, *in-situ* conservation and augmentation devices for parasitoids, and the self-propagation of entomopathogens.

Unlike classical and inundative biological control, CBC is often cost-effective; it may even enhance the other two BC strategies. It's been argued, for instance, that the failures of various classical BC programs stem from the absence of alternate hosts to sustain newly introduced beneficial organisms during specific periods (Gurr and Wratten, 1999). Could this be the case for *T. radiata*?

Should agroecosystem conditions favor the establishment of beneficial organisms, IBC could also be bolstered with fewer releases. The proposition integrating classical, inundative, and conservation biological control has been dubbed "integrated biological control" (Gurr and Wratten, 1999). Citrus cultivation in Mexico could serve as an initial model for integrated biological control.

Enhancing the biological control of *D. citri*

Inclusion and conservation of nectariferous plants. Due to prevailing crop conditions, the parasitoid *T. radiata* is necessarily absent from citrus for much of the year. One strategy for its conservation involves incorporating nectar-bearing plants to attract beneficial insect populations. However, not all nectar-bearing species are equally attractive, necessitating specific studies. For instance, Irvin *et al*. (2021), after evaluating four flowering plant species for the conservation of *Allograpta obliqua* (Diptera: Syrphidae), found that only two were attractive to the key predator of *D. citri* in California. Orchards with floral plants present recorded 2.5 times more predators and 3.5 times more oviposition than those without.

When fed with nectar from *Fagopyrum esculentum*, the survival of the parasitoid *T. radiata* significantly increased, and females laid five times more eggs than those fed with other sugar sources. Thus, the addition of nectar-bearing plants in citrus orchards could enhance *T. radiata* efficiency (Irvin and Hoddle, 2021). In northwest Michoacán, Mexico, out of over 27 species of flowering plants evaluated, only 24 registered parasitoids. Annual plants like *Stevia serrata*, shrubs like *Senecio salignus* and *Budleja* sp., trees like *Thouinia villosa*, and climbers like *Serjania racemosa* stood out. Over 14 families of parasitic hymenoptera were recorded on the flowers of these plants (Unpublished data).

The addition of nectar and pollen alone does not guarantee the prevalence of beneficial insects in agroecosystems. In the absence of prey or hosts for beneficial insects, they, being entomophagous, will be unable to complete their life cycle or will venture away in search of food, particularly parasitoids like *T. radiata*. Thus, the conservation of alternate hosts is essential for the persistence of beneficial insects alongside agroecosystems.

Conservation of Alternate Hosts. An example similar to *D. citri* was the biological control of *T. erytreae* using the parasitoid *T. dryi* in Trinidad Island. *Trioza eastopi* feeds on the weed *Litsea chinensis* (Lauraceae) and serves as an alternate host for the parasitoid, which managed to increase its population and successfully control the pest (Duran-Vila and Bové, 2015).

The species *Asclepias curassavica* (Apocynaceae) hosts specialist phytophagous (>9), including orders Hemiptera, Lepidoptera, Diptera, Coleoptera, and subclass Acarina. It encompasses species such as *Aphis nerii*, *Danaus plexippus* (Lepidoptera: Nymphalidae), *Euchaetes antica* (Lepidoptera: Erebidae), *Liriomyza asclepiadis*

(Diptera: Agromyzidae), and the mite *Eotetranychus typhae* (Tetranychidae), among others (Figure 2) (Cortez-Madrigal *et al.,* 2016).

In field conditions at Cienega de Chapala, Michoacán, specialized phytophagous of *A. curassavica* recruited over 20 species of natural enemies in citrus orchards, including egg parasitoids, aphids, and leaf miners, as well as a wide diversity of predators, including families Coccinellidae, Syrphidae, Chamaemyiidae, Chrysopidae, Staphylinidae, Cecidomyiidae, and orders Thysanoptera, Hemiptera, and mites of the family Phytoseiidae, among others (Figure 2) (Cortez-Madrigal *et al*., 2016). Among the recorded ladybird beetles, *O. v-nigrum*, *H. convergens*, and *C. sanguinea* are significant predators of *D. citri* (Michaud, 2004; Cortez-Mondaca *et al.*, 2010).

Studies conducted in citrus orchards recorded a significant reduction in pests of Persian lime cultivation, *Citrus x latifolia*, when the plant *A. curassavica* was incorporated, including *D. citri.* One explanation provided was the abundance of natural enemies recruited by *A. curassavica*, primarily from the families Coccinellidae, Syrphidae, Chamaemyiidae, and Chrysopidae (Godoy-Ceja and Cortez-Madrigal, 2018).

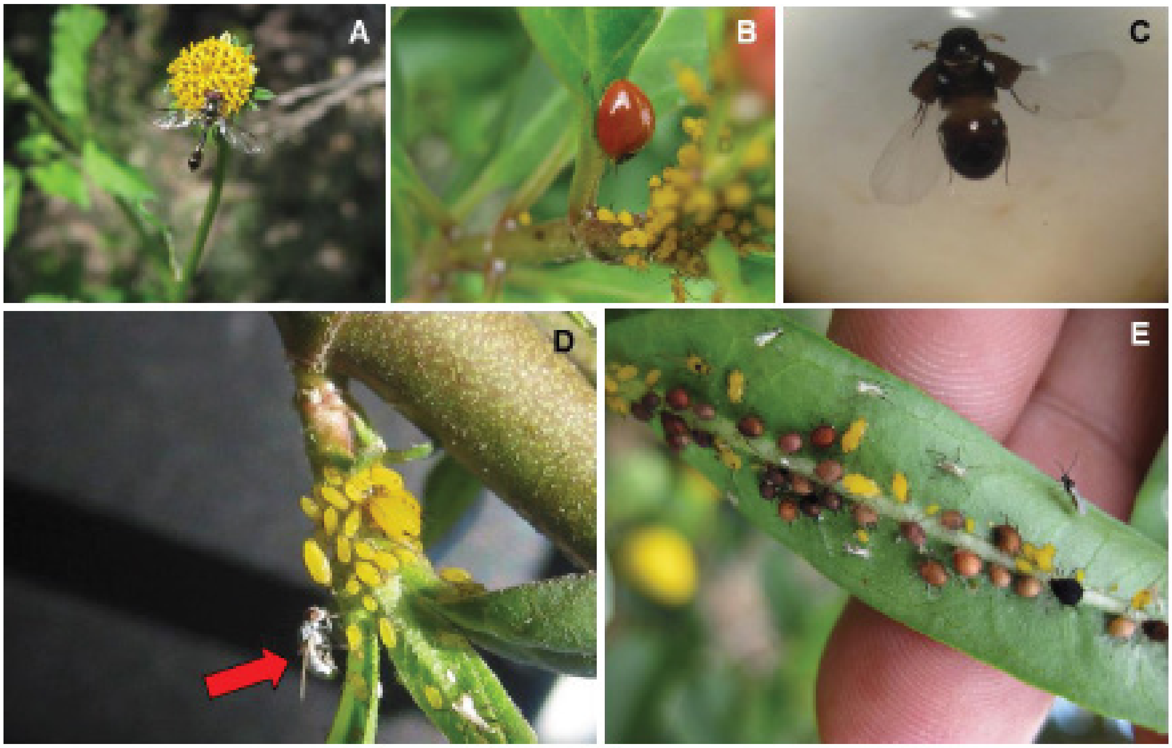

Figure 2. *Aphis nerii* on *Asclepias curassavica* and some associated natural enemies. A) *Pseudodorus clavatus*, B) *Cycloneda sanguinea*, C) Adult of *Oligota* sp., D) Adult of Chamaemyiidae, E) Mummies and adults of *Lyciphlebus testaceipes*.

The monarch butterfly emerged as a significant host for the egg parasitoid *Trichogramma pretiosum* (Trichogrammatidae), exhibiting multiple egg parasitism. In the study region, *T. pretiosum* was present for much of the year (Cortez-*Madrigal et al*., 2016), which could explain the low incidence of lepidopteran larvae pests in citrus (Godoy-Ceja and Cortez-Madrigal, 2018).

Devices for preserving parasitoids *in situ*. The objective is to conserve *in situ* parasitoids of sessile or low-mobility pests. In containers with emergence openings and selective mesh, infested plant material is deposited, allowing only the parasitoid's emergence (Kehrli *et al*., 2005). Another modification involves cages covering entire plants, adapted with emergence openings with selective mesh (Desurmont *et al.,* 2022), as mentioned earlier.

For the conservation of *T. triozae* (Hymenoptera: Eulophidae), a parasitoid of the tomato-potato psyllid (*Bactericera cockerelli*; Hemiptera: Triozidae), it was determined that a mesh opening of 500 µm allowed up to 89% parasitoid emergence and retained adult psyllids (Figure 3). In field conditions of tomato cultivation, the device promoted >80% parasitism of the psyllid (Unpublished data).

The same device with minor modifications could enhance the biological control of *D. citri*. For instance, through the design and use of cages similar to those described above, covering *Murraya* spp. plants with populations of *D. citri* and *T. radiata*. The vegetative characteristics of *M. paniculata* make it suitable

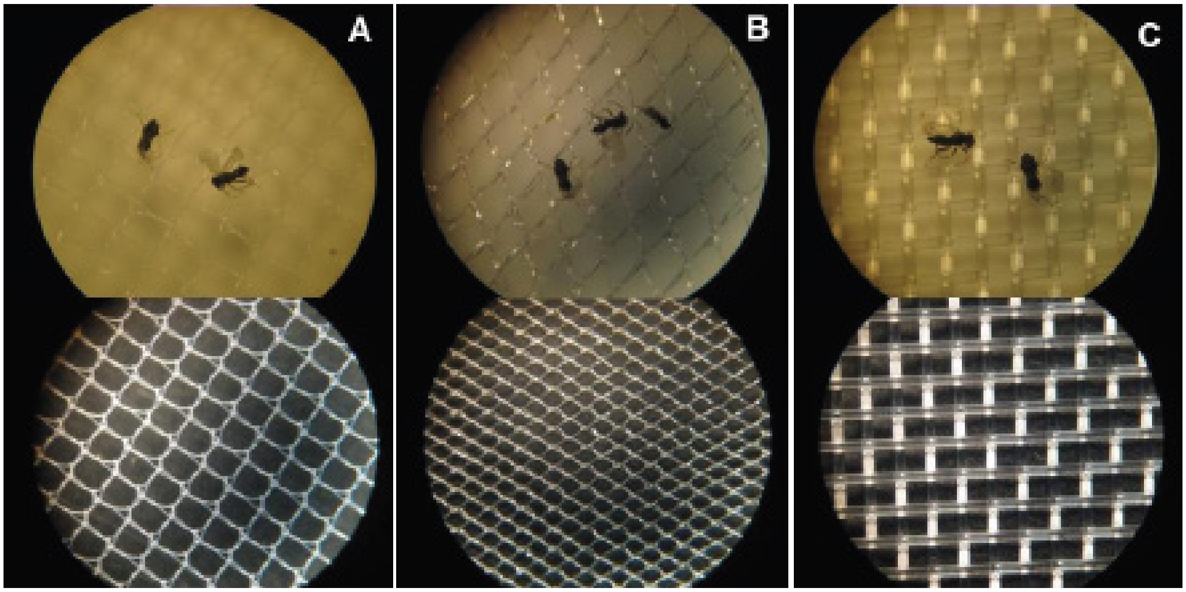

Figure 3. Different types of mesh evaluated in the selective emergence of *Tamarixia triozae*, parasitoid of *Bactericera cockerelli* as a model to be implemented with *D. citri* and its parasitoid. A) 700 x 700 µm, B) 700 x 900 µm, and C) 500 µm mesh.

for mass rearing programs of *T. radiata* for subsequent field release (Parra *et al*., 2016; García-Ávila *et al.,* 2021). In addition to *T. radiata*, the use of these devices could also conserve natural enemies of the psyllid. For example, during dry periods and absence of citrus flush in Yucatán, Mexico, *M. paniculata* hedges were where prevalence of *H. citriformis* was recorded (Cortez-Madrigal *et al.,* 2013).

Devices for the preservation and self-dissemination of EPFs. Some authors suggest the use of the self-dissemination technique of entomopathogenic fungi for managing *D. citri* (Avery *et al.,* 2010). However, few studies have been carried out. For instance, Patt *et al.* (2015) evaluated yellow devices baited with *I. fumosorosea* spores under greenhouse conditions. After 24 hours, 55% of adults were infected with the entomopathogen; when the mycosed cadavers were exposed to healthy nymphs, over 90% of them were infected. The blastospores on the trap remained viable for up to 20 days, which decreased when traps were directly exposed to sunlight. In another study using yellow cards impregnated with spores of the same fungus, adult mortality was less than 40%; when citrus volatiles were added to the cards, mortality increased by 23% (Moran *et al*., 2011).

For fungi species like *H. citriformis*, with low potential for inundative use, self-dissemination could favor the conservation and early development of epizootics. Epizootics of *H. citriformis* occur in *D. citri* populations in different parts of the world, with variable mortalities ranging from 75% in Florida (Hall *et al.,* 2012) to 92% in Rovirosa, Quintana Roo, Mexico (Mora-Aguilera *et al.,* 2017).

The self-dissemination of natural enemies could be facilitated by the behavior of *D. citri*, including mating with multiple partners (Wenninger and Hall, 2008), males' preference for mating with positive females, greater flight capacity of positive adults (Yamada *et al.,* 2019), and attraction to colored traps (Moran *et al*., 2011), among others. Additionally, the potential development of a sex pheromone by the insect could enhance the self-dissemination technique (Wenninger *et al.,* 2008).

Analysis and Insights

Despite the catastrophic forecasts since the appearance of HLB in 2009 (Salcedo-Baca *et al.,* 2010), efforts undertaken in Mexico have mitigated the worst impacts of the disease on Mexican citrus cultivation. However, eight years after its appearance, the disease continues to spread, and by 2017, records existed in all 24 citrus-growing states of the country (García-Ávila *et al.,* 2021). This underscores that the national citrus industry will always be at constant risk due to the presence of the *D. citri*-*C*Las pathosystem. Recent studies indicate that some Mexican citrus growers have opted to switch crops, which will result in a significant reduction in national citrus production (Granados-Ramírez *et al*., 2018).

It is clear that research on the *D. citri-C*Las pathosystem is far from concluded. Strategies are needed to reduce phytosanitary costs, where CBC plays a crucial role. The present review highlights the absence of studies and programs focused on conserving natural enemies for *D. citri*, despite demonstrating their importance for its management (Qureshi and Stansly, 2009; Irvin *et al.,* 2021). For instance, it has been estimated that in orchards without effective CBC programs, annual losses could reach up to 2,000 USD/ha (Monzó and Stansly, 2020). For reference, the cost of insecticide application in Florida citrus reached 3,000 USD/ha (García-Ávila *et al.,* 2021). Therefore, the long-term viability of the strategies implemented so far for HLB management should be reconsidered (Monzó and Stansly, 2020).

The strategies proposed here for CBC programs in citrus are straightforward and would not disrupt measures already implemented for managing the *D. citri-C*Las pathosystem. A future task is to evaluate *Murraya* species in conserving *T. radiata* by designing and using selective cages during periods of absence of *D. citri* nymphs in citrus. Besides protecting the parasitoid from pesticide applications, their populations would be advanced to synchronize them with the onset of nymph populations in citrus. Other studies demonstrate the feasibility of modifying the sequence of pest-NE appearance in agroecosystems to NE-pest, which is known to enhance BC (Doutt and Nakata, 1973; Jacas and Urbaneja, 2010).

There are numerous plant species that harbor specialized phytophagous insects that could serve as alternate prey for predators in citrus; that is, with low risk of becoming pests; an example is the Apocynaceae family (= Asclepiadaceae). In Mexico, the family is widely distributed with >300 species in the country (Juárez–Jaimes *et al*., 2007), thus presenting high potential for inclusion in *D. citri* CBC programs. In addition to *A. curassavica*, species such as *A. linaria, A. angustifolia*, and *Mandevilla foliosa* have recorded populations of *A. nerii*, *D. plexippus*, and Lygaeidae bugs. Even *C*Las-tolerant plants like *M. koenigii* could function as *D. citri* trap plants and harbor NE; among them, *T. radiata.*

In conjunction with alternate hosts, the contribution of nectar and pollen is crucial for attracting and sustaining a diverse array of predators that prey on *D. citri* (Kondo *et al*., 2015). However, the inclusion of nectar-producing plants in citrus crops for conserving D. *citri's* natural enemies remains an understudied aspect (Irvin *et al*., 2021; Irvin and Hoddle, 2021). Pioneering studies in Mexico, as described here, could serve as a foundational step for managing the pathosystem, especially in young citrus plantations where flowering is limited. Simultaneously, it's imperative to ensure that companion plants do not serve as hosts for *D. citri* or other significant pests.

D. citri's attraction to the yellow color presents opportunities for employing traps baited with entomopathogens, capitalizing on the insect's natural behavior for self-dissemination. The minimal inoculum required and the negligible impact on non-target organisms suggest that self-dissemination could serve as a valuable tool in CBC programs targeting the *D. citri-C*Las complex. One notable advantage of self-dissemination in vectors is its potential to trigger epizootics within populations (particularly in low-density populations), which could hypothetically slow down the spread and incidence of HLB. However, comprehensive field and laboratory epidemiological studies are needed to identify the biotic and abiotic factors conducive to epizootic development. This includes characterizing of EPFs, considering factors such as germination rates across various temperature and humidity conditions. Despite the common belief that high humidity is essential for the development of natural enemy pathogens (>90%; Tanada and Kaya, 1993), recent studies suggest that a humidity level as low as 53% could suffice for their germination and host mortality (Hastuti *et al*., 1999).

These findings open up possibilities for identifying more virulent isolates in adverse environments. For instance, citrus regions characterized by low macroenvironmental humidity but favorable microenvironmental conditions for EPFs (*e.g*., due to irrigation practices) could be potential hotspots for identifying highly effective isolates. Surprisingly, among 28 articles related to EPFs in *D. citri,* none address the isolate's response to humidity, indicating a significant gap in the existing research.

The assessment of other natural enemies, such as entomopathogenic nematodes (EPNs), remains a crucial area for exploration. Initial observations suggest that *Heterorhabditis indica* can infect and kill adult *D. citri* (unpublished data). However, the application of EPNs via foliage spraying has yielded limited results thus far. Hence, a pertinent question arises: how can EPNs be effectively applied for *D. citri* control? One plausible strategy could involve deploying attractive traps baited with juvenile EPNs. The swift mortality inflicted by these organisms on their hosts makes them an appealing tool for managing disease vectors like *D. citri.*

The vast diversity of the Mexican citrus agroecosystem poses a challenge for region-specific management of the *D. citri-C*Las pathosystem (Mora-Aguilera, 2013). Nevertheless, local management strategies applied to small and scattered citrus plantations, including ornamental Rutaceae, have demonstrated efficacy in disease control (Uechi *et al.,* 2019). These successful practices should be considered for integration into CBC programs in Mexico, aiming to promote crop diversification. For instance, the utilization of windbreak barriers, already recommended for mitigating vector and HLB spread (Stelinski, 2019), could be enhanced by selecting barrier trees with nectar-producing potential. This approach would offer dual benefits: serving as physical barriers and attracting natural enemies and pollinators.

In summary, the effective and sustainable utilization of natural enemies in CBC programs necessitates robust ecological and epidemiological studies. It's imperative to recognize natural enemies as integral components of complex ecological networks, contributing to intricate ecological-epidemiological interactions. The cultivation and spontaneous vegetation within agroecosystems play pivotal roles as the foundational trophic level, where various organisms, including entomophagous, entomopathogens, phytophagous, and phytopathogens, intersect. From these interactions emerge complex positive and negative feedback loops at each trophic level, ultimately influencing the health of citrus crops. For example, the predator-parasitoid-entomopathogen (P-P-E) complex associated with *Toxoptera citricida* indirectly promotes the survival of *D. citri* and thus *C*Las, by suppressing competition between the two pests for vegetative shoots. Ultimately, the entire D-P-E complex surrounding *D. citri* and *T. citricida* contributes positively to overall citrus health (Figure 4) (Mora *et al*., 2017).

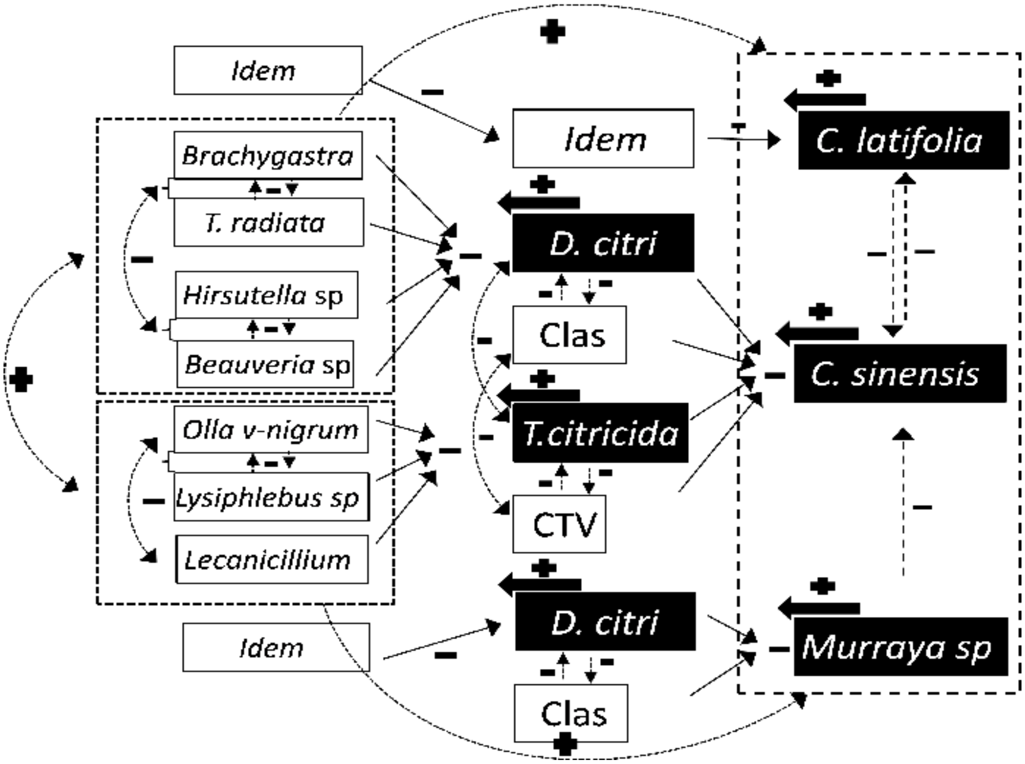

Figure 4. Phytosanitary trophic system in two citrus species (Persian lime and sweet orange) and the lemon grass *M. paniculata*, which are differentially infested with two pest-vector species (*D. citri* and *T. citricida*) and two pathogens (*Citrus tristeza virus* and *Candidatus* Liberibacter asiaticus). Taken from Mora *et al.* (2017).

CONCLUSIONS

The primary control strategy for *D. citri* and *C*Las involves insecticides, along with inundative biological control using *T. radiata* and some species of EPFs in a complementary manner. Although natural epizootics of fungi in *D. citri* occur, there is a lack of ecological and epidemiological studies aiding in the rational selection of EPFs isolates. Few studies have been conducted to expand their use endophytically and through self-dissemination.

Predators of *D. citri* are recognized as one of its main regulators, sometimes even surpassing *T. radiata* in various parts of the world. However, their extensive diversity has been underutilized in managing the pathosystem, mainly due to chemical control interference and the ecological simplicity of cultivation.

The absence of studies on conservation biological control programs for *D. citri* is evident, despite repeated mentions of its importance. Implementation of such programs could enhance not only the biological control of *D. citri*-*C*Las but also the self-regulation of citrus pests in general. A greater effort and understanding are required from all involved sectors: institutions, government, researchers, technicians, producers, and society at large. The challenge is substantial, but the vast diversity of regions and citrus production systems in Mexico could facilitate what would be a first step towards the concept of integrated biological control: through conservation, classical, and inundative approaches.

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